B-cell co-receptors: **The two-headed antigen**

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Lymphocytes often recognize antigens using not only their antigen receptors but also 'co-receptors' that bind other molecules associated with the antigen; the co-receptors then modulate the response to antigen. This concept has been used to make chimeric antigens that are extremely potent inducers of antibody responses.

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The immune response is initiated when an antigen is recognized by specific lymphocytes. Originally, 'antigen' was viewed as a simple entity that bound directly to lymphocyte antigen receptors: the B-cell antigen receptor complex that includes membrane-bound immunoglobulin, or the T-cell antigen receptor. It has been clear for a number of years, however, that conventional T cells recognize not free antigen, but rather antigen-derived peptides bound to proteins of the major histocompatibility complex (MHC). Now it is becoming clear that the antigen interacting with B cells may similarly interact with other molecules of the immune system that allow the B cell to gain additional information, affecting its response. In both T cells and B cells, the complexed antigen can bind not only the antigen receptor, but also a second cell-surface receptor referred to as the co-receptor. The complexed antigen brings together the antigen receptor and the co-receptor, with the result that antigen-receptor signaling events are altered and the cell responds accordingly. This principle has now been used to create antigens for B cells that are extremely good at stimulating the cells to generate an antibody response [1].

The ability of lymphocytes to gain important information about the recognized antigen was first appreciated in the case of T cells. Conventional T cells generally only become activated in response to antigen in the form of peptide-plus-MHC complexes. A key feature of the activation process is that the T cell can distinguish an MHC-bound ligand from other ligands because the T cell expresses cell-surface molecules, CD4 and CD8, which can bind directly to MHC proteins in domains distinct from their peptide-binding sites. Thus, the T cell recognizes the antigen as a complex ligand that binds to two of its cell-surface receptors — the T-cell receptor and either of two co-receptors, CD4 or CD8 — and brings the two together. The mechanism by which the T cell senses this dual interaction is not entirely understood, but one part of it involves Lck, a tyrosine kinase of the Src family. Lck is pre-bound to CD4 and CD8 and it phosphorylates key tyrosines in the cytoplasmic domains of various T-cell receptor subunits [2]. A distinct tyrosine kinase, ZAP-70, binds to the phosphorylated T-cell receptor chains and is then itself activated upon tyrosine phosphorylation; Lck is probably responsible for this activating phosphorylation. Lck also enhances T-cell responses by a second mechanism that does not require its kinase activity [3]. In the absence of CD4 or CD8 engagement, the T cell can still be stimulated, but typically this is much less efficient. In this case, it may be another Src-family member, Fyn — small amounts of which are bound directly to the T-cell receptor — that serves to initiate the early signaling events.

Until recently, the recognition of antigens by B cells was thought to be a simpler process, involving only an interaction between an antigen and the membrane immunoglobulin part of the B-cell receptor. But the concept is emerging that B cells also often recognize antigen in the form of a complex ligand which brings together the B-cell receptor and another cell-surface receptor, with important consequences. For example, it is now clear that if the antigen activates the complement system of proteins which, like lymphocytes, target invading pathogens — for instance, by causing the deposition onto the pathogen of complement protein C3b and subsequent breakdown products such as C3d — this not only enhances the overall immune response but also greatly enhances the response of the B cell itself. In this way, the adaptive immune response takes advantage of the ability of the 'alternative pathway' of complement activation to selectively target foreign entities, such as bacteria and viruses.

A dramatic example of this effect of complement protein C3d on B-cell activation has been provided by recent experiments of Dempsey *et al.* [1]. They created fusion proteins made up of the antigen chicken egg lysozyme and one, two, or three copies of C3d, a ligand for complement receptor 2 (CR2). When mice were immunized with these fusion proteins, their immune systems were strikingly more sensitive to the chimeric antigen than to lysozyme alone. Addition of one C3d moiety to the antigen resulted in 10-fold less antigen being needed to give rise to an equivalent antibody response; addition of two C3d moieties made the animals 100-fold more sensitive; and addition of three tandem C3d units made the animals approximately 1 000-fold more sensitive. This final fusion protein was an extremely effective antigen, even in the absence of conventional adjuvants, which are normally

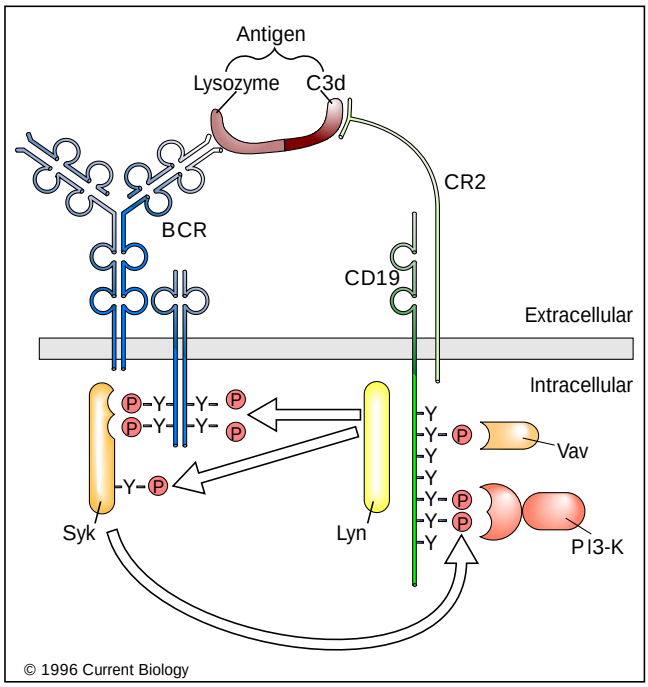
required to enhance the immune response to antigens: the response to this protein injected in saline was superior to that of lysozyme injected in complete Freund's adjuvant.

The ability of the fusion proteins to bring CR2 together with the B-cell receptor in the B-cell membrane is likely to be responsible for their impressive efficacy as antigens. The authors found that the fusion proteins were also more potent at inducing B-cell receptor signal transduction events. This result is consistent with earlier work in which cross-linking of CR2 with the B-cell receptor was found to enhance B-cell proliferation and signaling [4]. CR2 is present on the B-cell surface as a complex with other proteins: either complement receptor 1, or CD19, TAPA-1 (CD81) and Leu 13 [5]. Of these, CD19 appears to be primarily responsible for boosting signaling by the B-cell receptor, as co-cross-linking CD19 with the B-cell receptor potently enhances B-cell receptor signaling and B-cell proliferation, whereas co-cross-linking with other components of the CR2 complex is less effective [4]. The importance of CD19 is emphasized by the observation that B cells from mice lacking CD19, as a result of targeted disruption of the CD19 gene, have clear defects in their antibody responses, particularly to T-cell-dependent antigens [6,7]. Thus, the CR2/CD19 complex of B cells behaves like the CD4 and CD8 co-receptors of T cells in greatly boosting responses to antigen.

Two features of CD19 suggest possible mechanisms by which it could enhance B-cell receptor signaling (Fig. 1). Firstly, in human B cells, CD19 has been found to associate with Src-family tyrosine kinases, especially Lyn [8,9]. Thus, CD19 could enhance B-cell receptor signaling by a mechanism analogous to the primary mechanism by which CD4 and CD8 are thought to enhance T-cell receptor signaling upon engagement of peptide-plus-MHC ligands. In addition, B-cell receptor signaling induces prominent tyrosine phosphorylation of the cytoplasmic domain of CD19. This cytoplasmic domain has nine tyrosines, some or all of which may serve as phosphorylation-regulated binding sites for signaling components containing Src-homology 2 (SH2) phosphotyrosine-binding domains [4]. Indeed, two important signaling components, phosphatidylinositol 3-kinase and Vav have been shown to bind to the cytoplasmic domain of CD19 after stimulation [10,11].

So, antigens decorated with complement components serve to bring together the B-cell receptor and the CR2/CD19 co-receptor complex, and in this way greatly stimulate B-cell receptor signaling events. The B cell is also equipped to recognize a second type of complex ligand: an antigen molecule decorated with immunoglobulin G (IgG). This type of complex ligand brings together the B-cell receptor and FcγRIIB, a receptor for the constant part of IgG. In this case, rather than potentiating B-cell receptor signaling, the complex ligand attenuates it and so inhibits B-cell

Figure 1



Possible mechanisms for the co-receptor function of the CR2/CD19 complex in B cells. The co-receptor complex may function analogously to CD4 and CD8 in T cells, as it has been reported that CD19 associates with Src-family tyrosine kinases such as Lyn. According to this hypothesis, a complexed antigen or an artificial version of one, as used by Dempsey *et al.* [1] (as shown), would bring together the B-cell antigen receptor (BCR) and the CR2/CD19 complex, allowing Lyn to phosphorylate the B-cell receptor's cytoplasmic domain tyrosines and thereby creating binding sites for another tyrosine kinase, Syk. Syk appears to be activated by tyrosine phosphorylation, so Lyn could be responsible for activating Syk. Alternatively, the result of bringing together the B-cell receptor with CR2/CD19 by C3d-complexed antigen may be that tyrosine kinases bound to the B-cell receptor can now efficiently phosphorylate some or all of the nine tyrosines in the cytoplasmic domain of CD19. Some of these phosphorylated tyrosines serve as binding sites for the signaling components phosphatidylinositol 3-kinase (PI 3-K) and Vav.

activation. Presumably, this phenomenon is advantageous to the immune system because antigen–antibody complexes accumulate at a stage in the immune response when ample specific antibody has already been made and there is little need for resting B cells to become activated to produce more antibody. Co-engagement of FcγRIIB and the B-cell receptor interferes with B-cell receptor signaling by inducing the phosphorylation of a key tyrosine of the FcγRIIB cytoplasmic domain; once phosphorylated, this tyrosine serves as a binding site for the SH2-containing tyrosine phosphatase SHP (also known as PTP-1C and HCP) [12]. Presumably this phosphorylation is performed by a tyrosine kinase associating with the B-cell receptor, either a Src-family member (such as Lyn), or Syk, the B-cell homolog of ZAP-70. The key targets of SHP phosphatase action leading to decreased B-cell receptor

signaling and B-cell activation are not known at present. Nonetheless, what is clear is that B cells, like T cells, often see antigen not as a simple ligand for their antigen receptors, but rather as part of a complex with other immune system molecules that engage co-receptors and bring them together with the antigen receptors, with profound consequences for the response of the lymphocyte.

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